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Lipid oxidation and degradation products in raw materials: Low fat topical skin care formulations

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Abstract

Topical skin formulations with a lipid content below 15% were stored for six months at 5 ºC, 20 ºC, or 40 ºC or for 2 weeks at 50 ºC in darkness or at 20 ºC with exposure to light for six months . The volatile lipid oxidation compounds formed during this storage were compared to those formed in the raw materials during three months of accelerated stability storage at 40 °C for 3 months. The volatile compounds were collected by dynamic headspace and analysed by GC-MS.

It was possible to link eight out of nine volatile compounds detected during storage of topical skin formulations to the raw materials. In addtion, a possible link between the appearance of butane nitrile and the decomposition of an initiator used for polyacrylate crosspolymer-6 production was observed. The polymer may originate from texture modifiers added to the topical skin formulation or from plastics used for packaging of topical skin formulations.

Furthermore, six well-known lipid oxidation and non-enzymatic browning products were suggested to originate from the two raw materials, tricaprylin/tricaprin and coconut oil.

Introduction

In earlier studies, it was clearly demonstrated that lipid oxidation can occur in topical skin formulations with either high or low lipid content and that their sensory quality can be affected by lipid oxidation as changes in both odour and colour were observed [1-6]. It is tempting to assume that the extent of lipid oxidation will decrease with decreasing lipid content. However, this may not always be the case in "real" topical skin formulations because factors other than the absolute lipid content may be more important. Similar to other emulsions, topical skin formulations consist of three phases: the lipid and aqueous phase and an interface. Lipid oxidation is affected by partitioning and diffusion of anti-oxidants and pro-oxidants in all three phases and this can significantly influence lipid oxidation. Physical factors such as viscosity and droplet size may affect diffusion of pro-oxidants and, thereby, increase or decrease lipid oxidation [7].

Topical skin care formulations are complex systems consisting of many different ingredients, which may affect lipid oxidation positively or negatively. In addition, interactions between ingredients could influence lipid oxidation as also observed for simple emulsions [8]. However, only a limited number of studies on this topic are available in the literature. An earlier study of the skin conditioning raw material, coconut oil, showed that its peroxide value (PV) increased from approximately 2 to 65 meq/kg during 42 days of storage at 60 °C [9]. PV measures the primary oxidation products, which are odourless. Hence, PV cannot be used to asses odour changes in raw materials as a result of lipid oxidation. Odour changes are caused by secondary volatile oxidation products which were not measured in the above mentioned study.

Data on the quality of other raw materials used in topical skin care formulations are available in the literature [3, 9, 10]. However, the quality may vary widely depending on manufacturing process. This was shown in a more recent study, which compared the quality of coconut oils produced in India [10]. The authors concluded that the quality varied widely depending on crop quality and production method. They analysed three types of coconut oil; virgin coconut oil from wet mature coconut, and refined, bleached, and deodorized and unrefined coconut oil prepared from copra. Furthermore, they measured oxidative status by PV, which fluctuated from 0.0 to 2.7 meq/kg depending on manufacturer. In addition, the colour (0.00 – 2.7 Lovibond unit), free fatty acid (0.01 – 2.02 %), polyunsaturated fatty acid (0.13 – 1.57 %), monounsaturated fatty acid (3.31 – 5.23 %), saponification value (239.9 – 260.2) also varied widely between the production methods. The conclusion was that virgin coconut oil had the best quality independent of manufacturer [10]. These

studies support the notion that raw materials used for topical skin formulations may vary widely in quality because of their manufacturing process and that attention therefore must be paid to this fact when selecting raw materials for the manufacture of such products.

In an earlier study, we investigated lipid oxidation in raw materials used for production of a topical skin formulation with a high lipid content. We found that the most dominating volatile compounds clearly orginated from specific raw materials [1].

Several studies have explored the effect of lipid content on the oxidative stability in oil-in-water emulsions. One of these studies investigated the effect of pH and emulsifier type in two emulsions with a lipid concentration of 5 % and 70 %, respectively [11, 12]. In general, the study showed that the oxidative stability of the 5 % lipid emulsion was lower than the oxidative stability of the 70 % lipid emulsion. The lower oxidative stability was independent of emulsifier type. The findings were surprising as more oxygen can be dissolved in oil than in water. It was suggested that the accessibility to direct interaction of lipid hydroperoxides with the prooxidant trace metal ions in the aqueous phase was lower in the emulsions with high lipid content [11, 12, 13]. Aligned with our previous studies [11, 12], another study compared the oxidative stability of 10 % vs 30 % oil-in-water emulsions [13]. The oxidative stability was assessed by PV and anisidine value (AV) of the emulsions. The PV and AV were significantly affected by the lipid content, the 10 % emulsions had significantly higher amounts of hydroperoxides and aldehydes after 15 days storage compared to the 30 % emulsions. Overall, a decrease in the lipid content resulted in increased lipid oxidation [13]. The lower oxidative stability in these three studies may be related to low concentration of oil and thereby higher concentration of water that contains the prooxidant trace metal ions. Trace metal ions are well-known initiators of lipid oxidation in the initiation stage but also in the decomposition of hydroperoxides to secondary oxidation products. However, other factors than the oil content and presence of trace metal ions can also affect the oxidative stability. For example differences in oil droplet size could have affected the oxidative stability.

Before we are able to understand interactions between the raw materials in topical skin formulations, the oxidative status of the raw materials must be explored. The hypothesis of this study is therefore that oxidation occurs to a higher extent in low fat than in high fat topical skin care formulations and that it is possible to link the volatile compounds to the raw material(s) used in the formulations.

The purpose of this study was thus to measure lipid oxidation and oxidative degradation products in topical skin formulations with a lipid content below 15 % as well as in selected raw materials. A second aim was to investigate the link between volatile compounds affecting quality in topical skin formulations to the presence and formation of the same volatile compounds in raw material(s). In addition, we aimed at obtaining an understanding of the mechanism leading to the formation of certain volatile compounds. We used refined coconut oil and medium chain tricaprylin and tricaprin lipid sources in the formulations.

Lipid oxidation was accelerated by increasing temperature or light exposure to more quickly generate potential volatile compounds.

Materials

Prototype skin formulations

The topical skin care formulations used in this study were produced by GlaxoSmithKline (Brentford, United Kingdom). Two types of topical skin formulations were included in this study due to their different purposes and therefore different raw materials; 1) a prototype cleansing formulation (PCF) that contains rinsing agents to clean the skin. 2) a prototype serum formulation (PSF) that has a higher concentration of performance ingredients than other topical skin formulations with a low lipid content. It is used for targeting specific skin care concerns. The PCF contained several raw materials including glycerine, tricaprylin and tricaprin, coconut oil, lecithin and polyacrylate crosspolymer-6. The PSF contained several raw materials including glycerine and lecithin. Manufacturing protocols for the formulations are proprietary information.

Raw materials

Tricaprylin and tricaprin having the commercial name “Caprylic/capric triglycerides” 100 % (BASF SE, Ludwigshafen, Germany), Glycerine 99.5 % (Croda Europe Ltd, East Yorkshire, England), Coconut oil 100 % (Henry Lamotte oils, Bremen, Germany), Lecithin 88.2 % (Lipoid, Ludwigshafen, Germany), Polyacrylate crosspolymer-6 92-100 % (Seppic, Paris, France)

Methods

Storage experiment

PCF (in full 200 ml plastic bottles) and PSF (in full 30 ml plastic bottles) were stored for six months at 5 ºC, 20 ºC, and 20 ºC with exposure to artificial light (approximately 3500 lx), 40 ºC, and for 2

weeks at 50 ºC with sampling points after 0, ½ (only 50 ºC), 1, 2, 3 and 6 months. Individual containers were removed at each time point: one bottle of PCF and 3 bottles of PSF.

Raw materials were stored at 40 ºC for 3 months. Samples were taken each month (0, 1, 2 and 3 months). This storage condition was used to accelerate degradation and oxidation fast to reveal which volatile compounds that a certain raw material may give rise to. Furthermore, the concentrations in raw material and finished products are not to be compared directly

The samples were stored at 5 ºC until PV and GC-MS analysis. The product odor was assessed by an expert panel consisting of scientists in the R&D department at GSK at the end of the storage period. The scientists have expertise in using the degree of Difference (DOD) scale which they use at GSK to assess odor changes during storage. In this method, the sample odour was graded versus a reference. All samples are ranked from one to five. All samples ranked below 4 are within "product range".

Oil extraction

Oil was extracted from PCF and PSF with Bligh and Dyer method using a reduced amount of solvent [14, 15]. The method is described in more details in [1]. In brief, the water-soluble parts were separated from the lipid soluble parts by addition of chloroform, water and methanol followed by centrifugation. The lipid phase was used as starting material for PV analysis and determination of fatty acid composition.

Peroxide Value

PV was determined spectrophotometrically at 500 nm using the IDF method [16].

Quantification of volatile compounds

The volatile compounds were selected based on a prescreening, which considered their presence and increasing concentration in PCF and PSF during storage. The volatile compounds that appeared in PCF and PSF are different because they are produced from different raw materials.

Purge and trap on PCF

Extraction of volatile compounds and GC-MS analyses were performed as described by Thomsen et al. [17] for emulsions. In brief, the volatile compounds were released by continuously disturbing the equilibrium between the sample and headspace by purging nitrogen directly through the sample. The volatile compounds released from the sample were absorbed on a tube containing Tenax GR. After collection, the Tenax tube was manually inserted into an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, CT, USA), which transferred the volatile compounds from the Tenax tube to a focusing cold trap (-30 ^{o}C). Thereafter, the volatile compounds were transferred to the GC (Agilent 5890 IIA model Palo Alto, CA, USA) equipped with a DB1701 column (30 m × ID 0.25 mm × 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) using helium gas flow (1.3 mL/ min). The GC was connected to MS HP 5972 (Palo Alto, CA, USA) for analysis.

TDU/DHS on PSF and raw materials

Extraction of volatile compounds and GC-MS analyses were performed automatically using thermal desorption unit/dynamic headspace (DTU/DHS) as described by Thomsen et al. [17] with the following modifications for sample preparation, collection and water evaporation (Table 1). The extraction modification was performed in order to avoid contamination of the system and to remove water residues. Briefly, volatile compounds were automatically collected by purging the headspace (and not through the sample) followed by trapping the volatile compounds on the adsorbent tube using the Gerstel Tenax GR 300 tubes in a dynamic headspace station (Gerstel GmbH & Co. KG., Mülheim an der Ruhr, Germany). Then, the absorbent tube was automatically transferred by a thermal desorption unit/CIS (Gerstel GmbH & Co. KG., Mülheim an der Ruhr, Germany) into the GC 6890N Series –MS 5973 inert mass-selective detector (Agilent Technologies, Santa Clara, USA).

GC temperature program and MS settings for both purge and trap, and DTU/DHS

GC temperature-program: initial 45 °C for 5 min, 5 °/min til 90 °C, 4 °C/min to 220 °C and held for 4 min. The MS settings: electron ionization mode, 70 eV, mass to charge ratio (m/z) scan between 30 and 250.

<u>Fatty acid methyl esters (FAME)</u>

Fatty acid compositions of coconut oil, tricaprylin and tricaprin were determined in accordance with the method by Safafar *et al*. [18]. The analysis was conducted on 0.3 g of oil. Then, to the oil was added 100 µl internal standard 23:0 together with 200 µl heptane with BHT, 100 µl toluene and 1ml borontrifluoride in methanol. Samples and reagents were mixed and methylation was performed in a microwave oven at 100 °C for 5 min (Microwave 3000 SOLV, Anton Paar, Ashland, VA, USA) and the methylated sample was cooled down to room temperature. Then, to the methylated sample was added 1ml saturated NaCl and 0.7 ml heptane with BHT. Phase separation occurred, and the lipid/heptane phase of the methylated sample was analysed with Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-WAX fused silica capillary column (10 m×0.1 mm, 0.1 µm; Agilent Technologies, Palo Alto, CA, USA), using helium as carrier gas and a flame ionization detector. The GC temperature program: initial 160 °C, 10.6 °C/min until 200°C and held for 0.3 min, 10.6°C/min to 220°C and held for 1 min, and 10.6°C/min to 240°C and held for 3.8 min. The individual fatty acids were identified by matching their retention times to those of authentic standards. The result was expressed as area % of total fatty acids having a chain length between C8-C24, however the values reported below C14 are estimations. Only individual fatty acids present above 0.5 % was included.

Statistics analysis

A two-way analysis of variance followed by a Bonferroni multiple comparison test was employed to evaluate significant changes in PV (duplicates) and volatile oxidation products (triplicates) during storage. The calculation was conducted using Graph pad prism version 6 (graph pad, La Jolla, USA).

Results and discussion

Lipid oxidation in products

In PCF, PV was low initially and remained below 1 meq/kg during six months at 5 °C, 20 °C, 40 °C and during two weeks at 50 °C (Figure 1A). It was not surprising that exposure to light increased PV significantly to 20 meq/kg after two months of storage. This pattern was observed in other studies [2, 4, 6]. GC-MS analysis of the volatile compounds confirmed that lipid oxidation only occurred to a limited extent. Several volatile compounds were present in low concentrations (below

odour detection threshold (ODT) value in water) and did not show any clear pattern during six months (data not shown). This was the case for 2-ethyl furan, pentanal,1-penten-3-ol, 3-methyl-1-butanol, hexanal, 1-hexanol, heptanal, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, nonanal and decanal.

Even though lipid oxidation only occurred to a low extent, the concentrations of six volatile compounds increased during storage namely butanal, butanenitrile, 1-pentanol (Figure 1B-D), pentanenitrile, hexanenitrile and octanenitrile (data not shown). Butanal was not present initially and increased only slightly but significant during six months storage to 11-12.1 ng/g at 5 °C, 20 °C and 20 °C with exposure to light (Figure 1B), respectively. When exposed to 40 °C a significantly higher concentration was obtained after six months (16.5 ng/g). 1-Pentanol increased significantly to approximately 7 ng/g at all conditions after 6 months storage (Figure 1D). Butanal and 1-pentanol are well-known lipid oxidation products [19, 20, 21]. In an earlier study, we determined ODT values for volatile oxidation products, which increased during storage in a prototype cleansing formulation. In general, we found that ODT values in a prototype cleansing formulation were above 70 ng/g [6]. The cleansing formulation in this study is a matrix comparable to the cleansing formulation used for the ODT study, but with some small differences, which did not disqualify the ODT values previously determined from being used in the present study.. Therefore, butanal and 1-pentanol most likely did not affect product odour as individual compounds, but they may contribute to a cocktail effect when present together with other volatile compounds.

Butanenitrile, pentanenitrile, hexanenitrile and octanenitrile were reported to be present in topical skin formulations [2] but the mechanism of their formation during storage was not explained. Butanenitrile has been described as having a bitter almond-like odour [22, 23]. The ODT value in water was 32 ng/g for butanenitrile [24]. The concentration of butanenitrile, pentanenitrile, hexanenitrile and octanenitrile increased significantly during storage at 20 °C with exposure to light and 40 °C. However, the concentrations were below 70 ng/g, they are not expected to affect odour as individual compounds.

Similar to PCF, PSF was also selected as a representative of topical skin formulations with low lipid content. PSF contains a higher number of skin conditioning raw materials compared to PCF.

For PSF, initially PV was 0.25 meq/kg and it remained below 0.3 meq/kg at 5 °C. It increased significantly after 6 months' storage to 1.0 meq/kg, 1.3 meq/kg and 2.5 meq/kg at the storage conditions 20 °C, 20 °C with exposure to light and 40 °C, respectively (Figure 2A). Again, the low PV may be related to a fast conversion of hydroperoxides to secondary volatile oxidation products. After 3 months' storage, most volatile oxidation compounds seemed to be formed almost simultaneously with the peroxides indicating that there was a lag period before oxidation took off after 2 months storage (Figure 2). Concentrations of several aldehydes increased in PSF during storage: butanal, pentanal, hexanal and benzaldehyde. Butanal, pentanal and hexanal are all well-known lipid oxidation products, whereas benzaldehyde has been suggested to arise from non-enzymatic browning reactions [19, 20, 25]. In addition to the aldehydes, the concentration of two ketones (2-pentanone and 2-hexanone) and one alcohol (1-pentanol) increased as well.

For the two short-chained aldehydes, butanal and pentanal, concentrations were initially low but increased significantly during storage at all conditions. After 6 months of storage, their concentrations increased significantly and above the ODT values at 130±10 ng/g and 100±6 ng/g for butanal and pentanal obtained in topical skin formulations with a low lipid content [6]. The ODT values were exceeded for PSF stored at 20 °C (only for pentanal), 20 °C with exposure to light and 40 °C. For butanal, the concentration increased to 154 ng/g and 141 ng/g in PSF stored during 6 months at 20 °C with exposure to light and 40 °C, respectively (Figure 2B). This concentration of butanal can affect the odour to become more cheese-like and citrus sour [6]. For pentanal, the concentration increased to 119 ng/g, 184 ng/g and 185 ng/g when stored during 6 months at 20 °C, 20 °C with exposure to light and 40 °C, respectively (Figure 2D). This concentration of butanal can affect the odour to become more green and acidic milk-like [6].

In contrast to the large increases observed in the concentrations of short chained aldehydes, concentrations of the two aldehydes with a longer chain, hexanal and benzaldehyde, only increased slightly to 69 ng/g (but significantly after 6 months at 20 °C with exposure to light and 40 °C) and 17 ng/g (Figure 2F and 2H). No significant increases were observed for the two ketones, 2-pentanone and 2-hexanone, for which their concentrations only increased slightly to 13 ng/g and 15 ng/g (Figure 2C and 2E). These low concentrations are not expected to affect product odour as individual compounds although they may contribute to a cocktail effect.

The alcohol, 1-octanol increased significantly during storage at 40 °C to 1803 ng/g after 6 months´ storage. At this high concentration, it is expected to affect product odour. At the other storage

conditions, 1-octanol also increased significantly after 6 months´ storage to 102 ng/g, 279 ng/g and 222 ng/g at 20 °C, 20 °C with exposure to light and 40 °C, respectively (Figure 2G). The exact ODT value for 1-octanol in topical skin formulation has not been determined, but the ODT value for 1-heptanol has been measured in topical skin formulation and was found to be 170±23 ng/g [6]. The volatility of 1-octanol is expected to be lower than 1-heptanol. Therefore, a slightly higher ODT value may be expected for 1-octanol than for 1-heptanol. Nevertheless, it is likely that the observed concentrations after 6 months (279 ng/g, 222 ng/g and 1083 ng/g) may affect product odour.

Comparison of PV results with PV data obtained in our earlier study [1], showed that the oxidative stability was lower for topical skin formulations having a low lipid content than in topical skin formulations with a high lipid content. The PV was up to 21.9 meq/kg and 1.44 meq/kg in topical skin formulations having a low and high lipid content, respectively. However, the concentration of volatile oxidation and degradation products revealed that the oxidative stability of topical skin formulations with a high lipid content was lowest. The same pattern was observed in this study namely that a low PV was related to a high concentration of volatile compounds, but a high PV did not result in a high concentration of volatile compounds. Consequently, no direct relationship was observed between PV and volatile compounds neither in the present study nor in the previous study. The concentration of butanal increased to 154.35 ng/g and 408.72 ng/g in topical skin formulations having a low and high lipid content, respectively. The higher PV for topical skin formulations with a low lipid content may be related to a faster conversion from primary to secondary oxidation products. The hypothesis that the low fat topical skin care formulations would have a lower oxidative stability than the high fat products could thereby not be confirmed. Studies in simple emulsions [11, 12, 13] obtained the opposite result namely that decreasing lipid content increased oxidation. The reason for these contradicting results may be related to effects on lipid oxidation from the numerous raw materials used for topical skin formulations e.g. addition of polymers, which may make it difficult to compare the effect of the lipid content in different types of products. Therefore, more studies are needed to investigate this issue.

Lipid oxidation in selected raw materials

A broad screening for volatile compounds in mainly lipid containing raw materials was conducted. The concentration of product relevant to volatile compounds increased notably in five of the raw materials used for the prototype skin formulations during 3 months´ storage, namely, glycerine and

lecithin applied in PCF and PSF, and tricaprylin and tricaprin, coconut oil and polyacrylate crosspolymer-6 for PCF only.

The main focus was on volatile compounds initially present and also appearing during storage both in topical skin formulations and raw materials. An explanation to the appearance of the volatile compounds in the raw material is suggested based on fatty acid composition (degree of unsaturation) and other studies reported in literature.

The raw materials; tricaprylin and tricaprin, glycerine and coconut oil contained several aldehydes (Figure 3). The concentration of volatile compounds increased particularly in tricaprylin and tricaprin, and coconut oil during accelerated storage at 40 °C for 3 months.

The increasing concentration of butanal in both prototype skin formulations may be related to two raw materials, tricaprylin and tricaprin and glycerine. The raw material, tricaprylin and tricaprin, also contained 1-pentanol and 2-pentanone after accelerated storage. Therefore, the increase in the concentration of 1-pentanol in PCF may be related to this raw material.
The three aldehydes, pentanal, hexanal and benzaldehyde, and one ketone, 2-hexanone, originated partly from three raw materials, tricaprylin and tricaprin, glycerine, and coconut oil. Several other volatile compounds increased in these raw materials but not in the products, namely 3-methylbutanal, 3-methyl-1-butanol, 2-heptanone, heptanal, octanal and nonanal. We hypothesize that this could be because the skin care product matrix highly influenced the release of volatile compounds. Furthermore, the concentration of the volatile compound detected in the neat raw material may be below the detection limit for GC-MS method when the raw material is mixed with other ingredients in the skin care product. However, 6 out of 9 volatile compounds that increased during storage in PCF also appeared and increased in the raw materials.
The increasing concentration of volatile compounds in tricaprylin and tricaprin can be related to its fatty acid composition. tricaprylin and tricaprin mainly contained fatty acids with a shorter chain length than C14. It contained the saturated fatty acids (>99 %; 57.02 % of 8:0 and 42.30 % of 10:0) and unsaturated fatty acids (< 0.5 %; 16:1 and 16:2). Even though the degree of unsaturation was low, tricaprylin and tricaprin oxidized to a large extent during accelerated storage. This may be due to the fact that monounsaturated fatty acids can undergo autooxidation at elevated temperature.

The humectant raw material, glycerine was a relatively stable raw material. As described previously [1], glycerine can oxidize to aldehydes such as glyceraldehyde, which may react with other

molecules through the mechanism described by Jungermann and Sonntag [26]. However, impurities in the raw material (0.5 %) may also contribute to the volatile compounds developing during accelerated storage.

The other raw material for which the concentration of volatile compounds increased during storage was coconut oil. Again, it can be explained by its fatty acid composition. Coconut oil mainly contained the saturated fatty acids (> 90 %; 7.65 % of 8:0, 5.81 % of 10:0, 45.68 % of 12:0, 18.29 % of 14:0, 9.89 % of 16:0, 2.87 % of 18:0). However, it also contained unsaturated fatty acids; mono-unsaturated (> 7 %; 7.30 % of 18:1 n-9) and polyunsaturated (< 2 %; 1.83 % of 18:2 n-6). The polyunsaturated fatty acids are highly susceptible to autoxidation, which may explain the large increase observed in the concentration of volatile compounds.

In addition to the lipid containing raw materials, other raw materials, which were present in a concentration above 1 %, were also screened for volatile compounds during accelerated storage. Amongst those, lecithin and polyacrylate crosspolymer-6 had increasing concentrations of at least 6 of the 9 products relevant volatile compounds (Figure 4).
After 3 months of accelerated storage, a broad variety of volatile compounds appeared in both raw materials. In lecithin and polyacrylate crosspolymer-6, the concentration of butanal, 2-pentanone (only lecithin), pentanal, 2-hexanone, hexanal and benzaldehyde increased. Therefore, these raw materials may partly contribute to the increasing concentrations observed in the topical skin formulations. Several other volatile compounds increased in the texture modifying raw materials, but not in the prototype skin formulations: 3-methylbutanal, 2-heptanone, heptanal, octanal and nonanal.
None of the raw materials contained butane, pentane, hexane or octanenitrile. However, a nitrile containing compound was found in polyacrylate crosspolymer-6, namely tetramethylbutanedinitrile. It was therefore speculated that butanenitrile appearing in the PCF during storage may be related to decomposition of the nitrile containing impurities in polyacrylate crosspolymer-6.
A literature search showed that other authors have studied the formation of butanenitrile (and other nitrile containing compounds), but most of the reactions suggested to lead to the formation of butanenitrile require high temperature above 176.85 °C [27]. The high temperature reaction conditions required exclude the reactions from taking place in this study as butanenitrile is appearing at low tempeture at room temperature. However, an alternative reaction route to butanenitrile may have occurred. Tetramethylbutanedinitrile has been suggested by other authors as

a by-product from polymer and plastic production. In polymer and plastic production, azobisisobutyronitrile (AIBN) is often used as an intiator of polymerisation. After AIBN has fullfilled its purpose in polymer and plastic production, it decomposes to form 2-cyanoprop-2-yl radicals or/and tetramethylbutanedinitrile as by-products (Figure 5). Several authors have reported isobutanenitrile as a secondary by-product from AIBN, but no authors have reported that they have detected butanenitrile [28, 29].

Since the formation of isobutanenitrile requires less energy than the formation of butanenitrile [30], it is suprising that this compound could not be detected, whereas we were able to detect butanenitrile. Another possibility is that butane, pentane, hexane and octanenitriles are migrants from plastic packaging. Since, butane, pentane, hexane and octanenitriles appear in prototype skin formulations stored in both plastic and glass packaging, migration does, however, not seem to be a plausable explanation. More studies are needed to fully understand the reaction routes leading to the detected nitriles.

Linking volatile oxidation products in PCF/ PSF and raw materials together

The volatile compounds both present in the prototype skin formulations, PCF and PSF, and in raw materials are summarized in Table 2. In brief, the increasing concentration of butanal in PCF and, especially, PSF during storage may originate from all the selected raw materials except coconut oil. However, the concentrations were conspicuously higher and above ODT value after accelerated storage in tricaprylin and tricaprin, and polyacrylate crosspolymer-6. Therefore, it is most likely that butanal originated from these two raw materials.

It is not surprising that pentanal increased in all raw materials. This was also the case in our previous study [1]. The concentration was above ODT value in all raw materials except glycerine after 3 months accelerated storage. The volatile aldehyde in PSF, hexanal, was present in all raw materials after 3 months accelerated storage. Especially, tricaprylin and tricaprin, and coconut oil had high concentrations of hexanal at 1681 ng/g and 3976 ng/g. Benzaldehyde increased in PSF to 17 ng/g after 6 months´ storage. It was possible to link benzaldehyde to all raw materials.

The appearance of butanenitrile was surprising; it is usually not observed in studies of lipid oxidation. A likely explanation to its appearance has been suggested, but more studies are needed to identify the exact route of formation from impurities in polyacrylate crosspolymer-6.

The alcohol 1-pentanol increased in one raw material, namely tricaprylin and tricaprin. The other alcohol that increased in PCF, 1-octanol, was not to linked to any of the raw materials. As observed previously [1], the two ketones, 2-pentanone and 2-hexanone, were only present in low concentrations in both PSF and raw materials.

Despite the product odour changing during storage in topical skin formulation, the product odour was still deemed within product range by an expert panel.

Therefore, it was possible to link eight out of nine volatile compounds found in the prototype skin formulations to raw materials. GSK Toxicology group has assessed the human safety impact of the volatiles included in this study. At the determined levels these substances do not raise any toxicological concern, neither locally or systemically.

Conclusion

This study explored lipid oxidation and oxidative degradation in two topical skin formulations (PCF and PSF) containing a low lipid content. In an earlier study, we investigated lipid oxidation and oxidative degradation in a topical skin formulation containing a high lipid content. Comparison of these two studies revealed that the oxidative stability measured by PV decreased with decreasing lipid content for topical skin formulations. However, the opposite was observed for the concentration of volatile oxidation and degradation products. Thus, the concentration of the volatile compounds was higher in the topical skin formulations with a high lipid content than in the topical skin formulations with a low lipid content. These results are contrary to those for simple emulsions. The higher stability of the topical skin formulations with a low lipid content may be related to their high complexity due to the large number of raw materials, which can affect lipid oxidation such as Polyacrylate crosspolymer-6. However, more studies are needed to investigate this difference between simple emulsions and topical skin formulations.

Similar to our previous findings for topical skin formulations with a high level of lipids, several secondary volatile oxidation products were present initially and more were formed during 6 months of storage.

Selected raw materials were explored in order to link volatile compounds affecting the quality in the topical skin formulation to raw material(s) and eight out of nine volatile compounds found in topical skin formulations could be linked to their presence in raw materials. Thus, well-known lipid oxidation products and non-enzymatic browning products found in PSF and PCF were suggested to

originate from tricaprylin and tricaprin, and in particular coconut oil because of its unsaturated nature.

Butanenitrile appeared during storage in PCF. This compound has not been reported in other lipid oxidation studies either in model emulsions, food emulsions or in topical skin formulations. Since the concentration of butanenitrile was low it most likely did not affect product odour, but it is still important to explore the mechanism behind its formation. A possible link between butanenitrile and the decomposition of the initiator used for production of polyacrylate crosspolymer-6 was identified. However, more studies are needed to determine the exact reaction route from this ingredient to butanenitrile.

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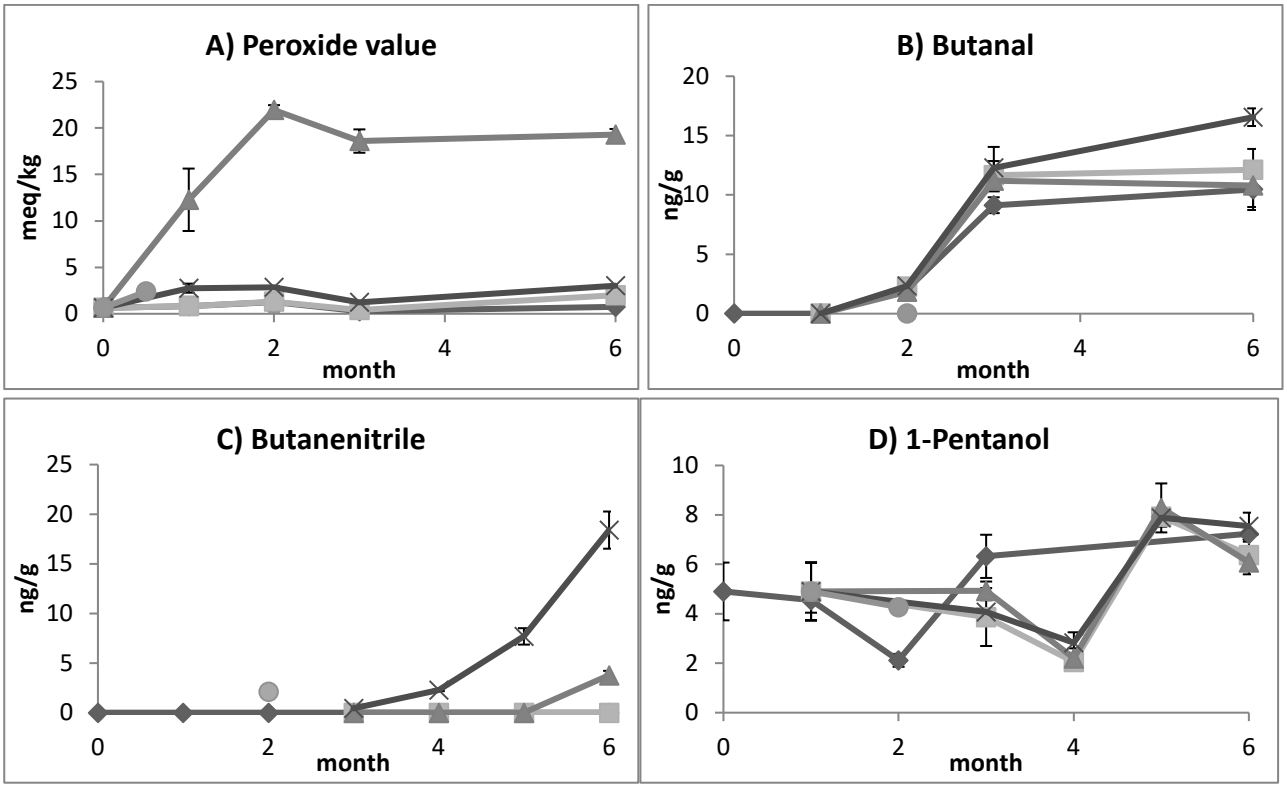

Figure 1. Lipid oxidation and degradation products in PCF during 6 months storage at 5 °C (◆), 20 °C (■), 20 + light (▲), 40 °C (×) and 50 °C (●). The development of A) Peroxide value in meq/kg, B) Butanal, C) Butanenitrile and D) 1-Pentanol in ng/g.

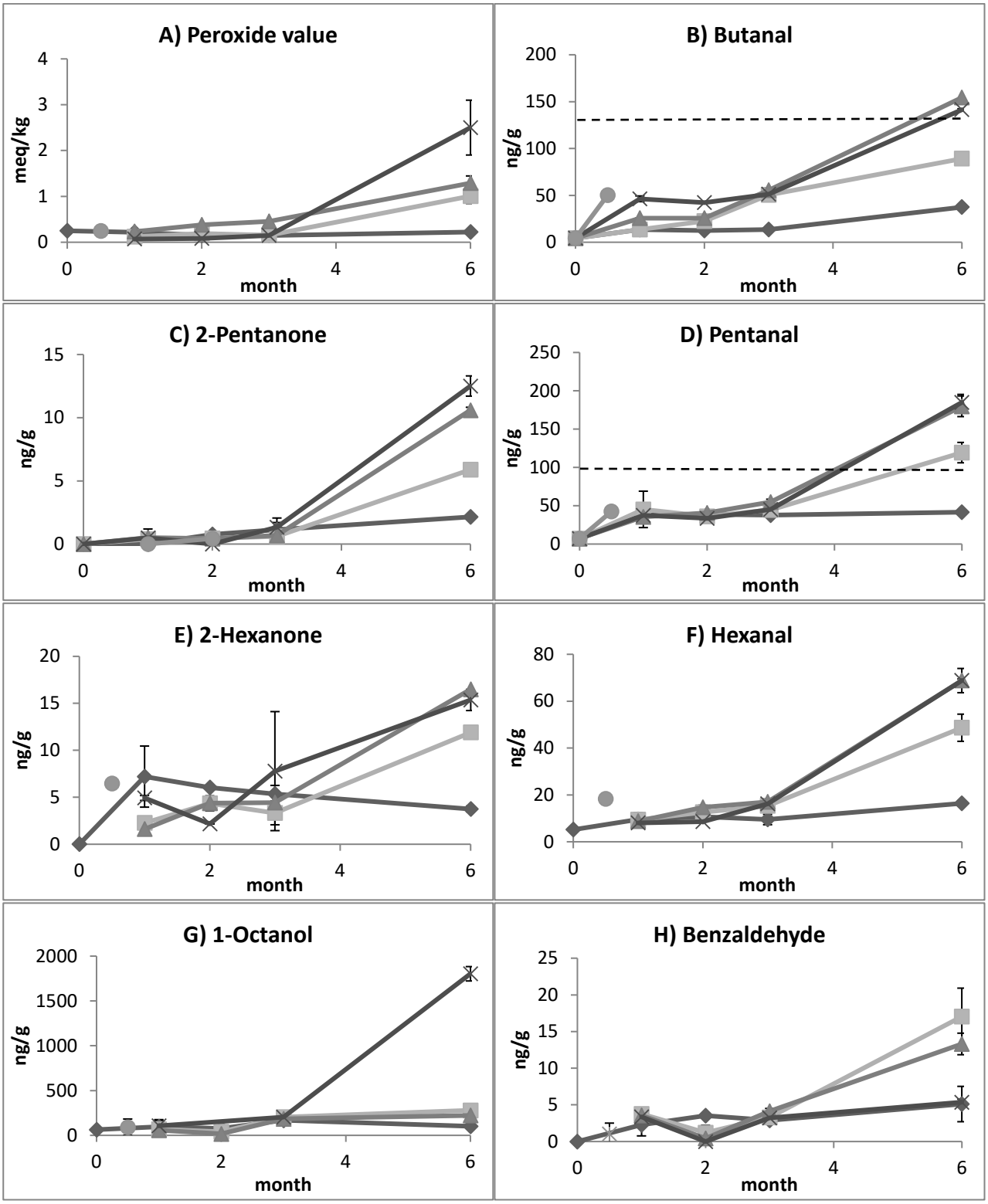

Figure 1. Lipid oxidation and degradation products in PSF during 6 months storage at 5 °C (◆), 20 °C (■), 20 + light (▲), 40 °C (×) and 50 °C (●). The dotted line indicates the exact ODT value (determined in product) (butanal and pentanal). The development of A) Peroxide value in meq/kg, B) Butanal, C) 2-Pentanone, D) Pentanal , E) 2-Hexanone, F) Hexanal, G) 1-Octanol and H) Benzaldehyde in ng/g.

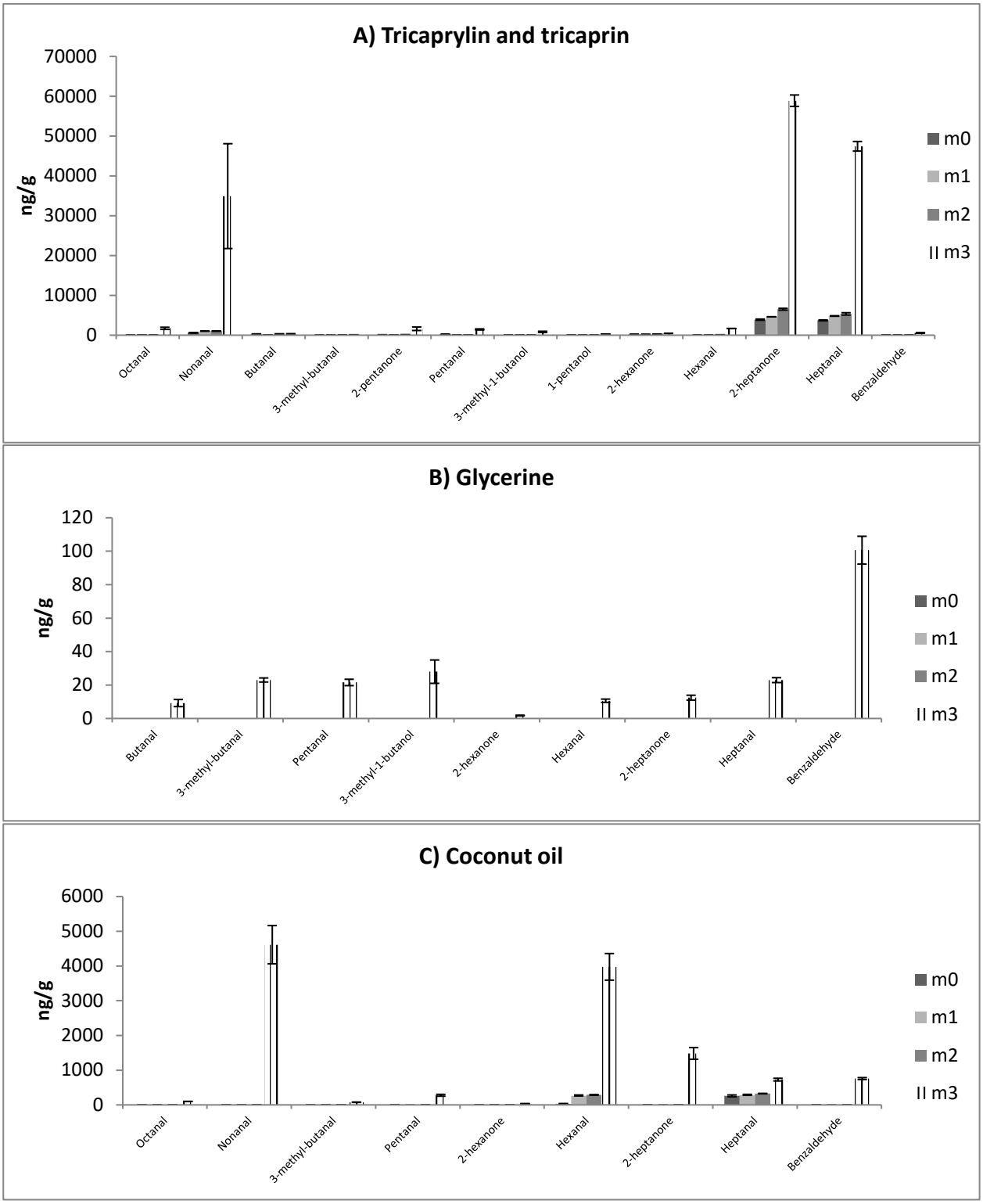

Figure 1. Volatile compounds [ng/g] present in skin conditioning and emollient raw materials during 3 months storage at 40 ºC. A) tricaprylin and tricaprin, B) glycerine, and C) coconut oil.

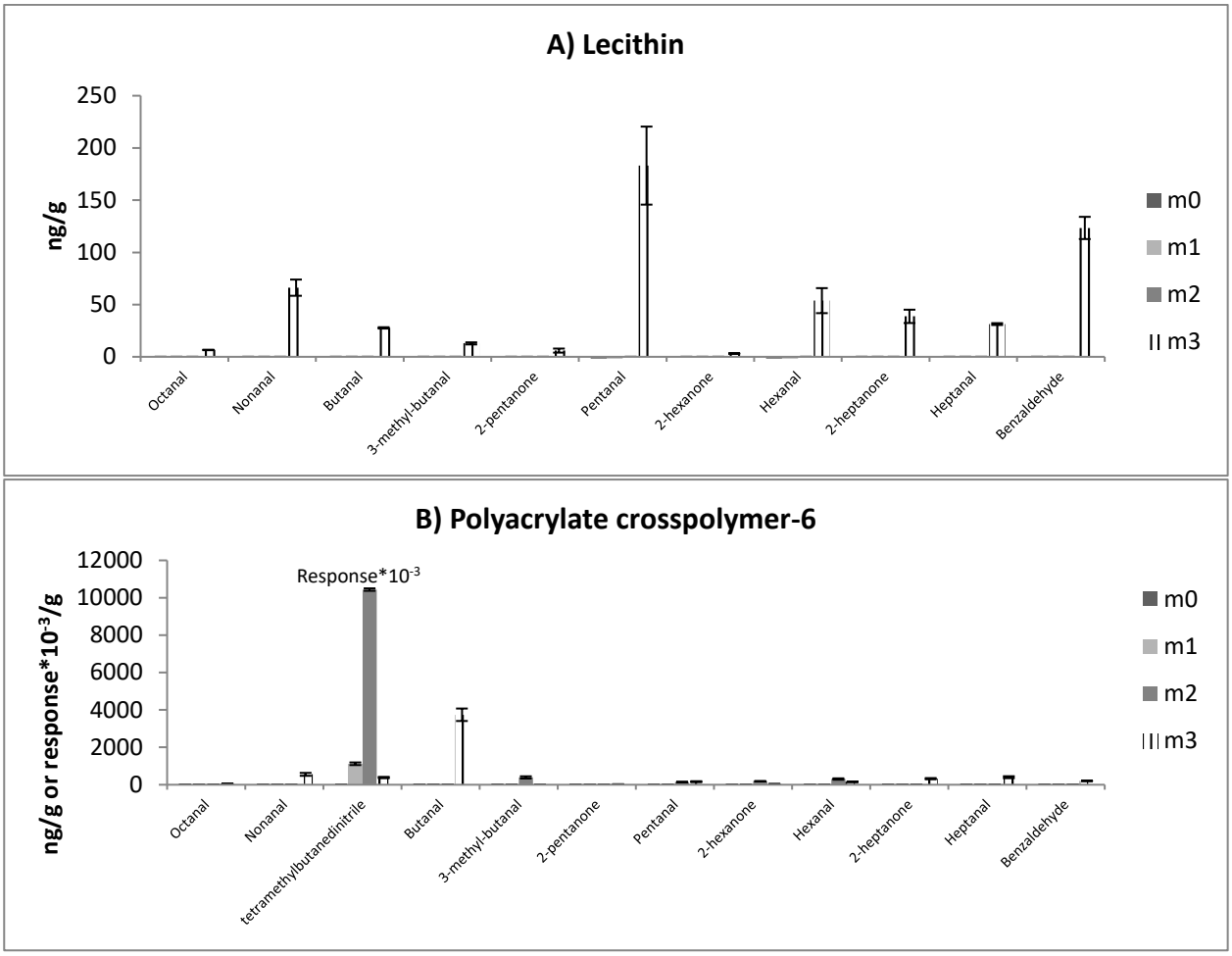

Figure 1. Volatile compounds [ng/g and response*10^{-3}/g(only tetramethylbutanedinitrile)] present in texture modifying raw materials during 3 months storage at 40 ºC. A) Lecithin and B) Polyacrylate crosspolymer-6.

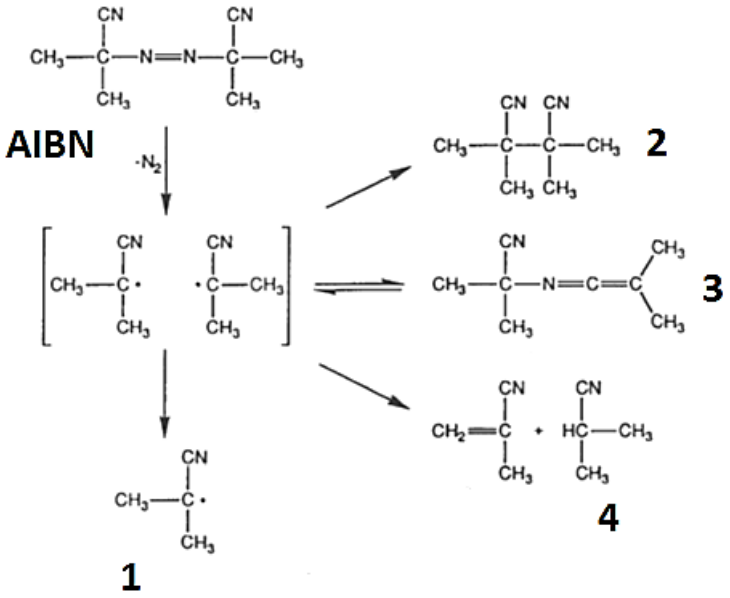

Figure 1. AIBN decomposes to form 1. 2-cyanoprop-2-yl radicals, 2. Tetramethylbutanedinitrile, 3. Dimethyl-N-(2-cyanoprp-2-yl)ketenimine and 4. isobutanenitrile. A modification of Krstina *et al*. [29].

Table 1. The modifications for sample preparation, collection and water evaporation

Samples	Preparation	Collection	Evaporation
PSF	1 g sample. Incubation at 45 °C for 5 min.	50.0 mL/min at 45 for 10 min	50 ml/min at 25 °C for 22 min.
Tricaprylin and tricaprin Glycerine Coconut oil	1 g sample. Incubation at 60 °C for 4 min.	50.0 mL/min at 60 for 20 min	-
Lecithin Polyacrylate crosspolymer-6	1 g of sample and water were mixed(1:1). Incubation at 45 °C for 5 min.	50.0 mL/min at 45 for 10 min	50 ml/min at 25 °C for 22 min.

Table 2. Summary of volatile compounds present in both products and raw materials. + = present, ++ = present above ODT value in raw material (only available for butanal and pentanal), and - = absent.

Volatile compounds/ Raw material	Butanal	Pentanal	Hexanal	Benzaldehyde	Butanenitrile	1-Octanal	1-Pentanol	2-Pentanone	2-Hexanone
Tricaprylin and tricaprin	++	++	+	+	-	-	+	+	+
Glycerine	+	+	+	+	-	-	-	-	+
Coconut oil	-	++	+	+	-	-	-	-	+
Lecithin	+	++	+	+	-	-	-	+	+
Polyacrylate crosspolymer-6	++	++	+	+	-(?)	-	-	+	+